IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty Dkt: LOESSNER-1

In re application of: Martin LOESSNER *et al.*	Confirmation No: 5123
Appl. No.: 10/516,507	Art Unit: 1651
Filed: December 1, 2004	Examiner: Satyendra SINGH
For: VIRULENT PHAGES TO CONTROL *LISTERIA MONOCYTOGENES* IN FOODSTUFFS AND IN FOOD PROCESSING PLANTS	

DECLARATION OF MARTIN LOESSNER PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Dear Sir:

I, the undersigned, declare as follows:

1. I am a co-inventor of the above identified patent application and am familiar with its contents. I currently hold a Chair position as a Professor of Food Microbiology at the Institute of Food Science and Nutrition at the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland. I have worked in the field of microbiology and molecular biology, with an emphasis on food-borne pathogens and their specific bacteriophages, for more than 20 years. I have published over 60 scientific papers in this field, am an inventor of several patents relevant to this field, and have authored or co-authored several book chapters and a popular textbook on Food Microbiology. My work has been internationally recognized and has received several awards. In addition, I am a member of the Bacteriophage Subcommittee of the International Committee on Taxonomy of Viruses (ICTV), and the Swiss National Delegate to the International Committee on Food Microbiology and Hygiene (ICFMH).

2. I have read and understand the current Office Action and the references cited by the Examiner in the rejection of the pending claims. My initial purpose here is to discuss the reference U.S. Patent 5,006,347 of Day *et al.* ("Day"), but also to comment on the two additional references

that are papers published by me and my colleagues. It is worth noting that the focus of Day is the treatment of milk used for cheese making. I note that all the claims concern only cheese and no other foods. Day is focused on the use of phages to remove bacteria of the genus *Clostridium* (which cause spoilage), though *Listeria* is mentioned most briefly, in passing. Indeed, the word "*Listeria*" appears only twice, once at col. 2, line 65 and once in claim 4.

3. At the time of the Day disclosure (1989) and its priority application (1987), however, the very *existence* of true virulent (strictly lytic) *Listeria* phage[1], and particularly, P100 of the present invention, *was unknown* and could by no means have been anticipated. I and my colleagues have since shown (see attached reference describing work by me, co-inventor Carlton and colleagues,[2] after the making of the present invention) that temperate phages, which had been described in the older literature, are unsuitable for the purpose of killing and thereby removing *Listeria* organisms from food, food processing equipment or food containers. The Carlton paper lists and explicitly discusses the various arguments against the use of temperate phages in this context.

4. Our discovery of the P100 phage was first disclosed in the priority document of the present application (priority date of July 2002) as a new and innovative discovery. The phage was deposited at the ATCC in a patent deposit accorded accession number PTA-4383. Therefore, P100 could not have been described (nor could its existence have been suggested) six or twelve years earlier in the two papers[3] by me and my colleagues that were cited as (a) anticipating some of the present claims, and (b) in combination with Day in an obviousness rejection. Clearly, we (and the world) did not yet know of the existence of phage P100 when we conducted the research and wrote these two papers. Furthermore, phage P100 is a distinct entity from the A511 phage (deposited at the ATCC as accession number PTA-4608). A511 was known before the present invention was made, and was discussed, for example, in the cited Loessner-I and Loessner-II papers. P100 differs substantively from A511 both in its host range as well as on the basis of its genome sequence and structure. At this time both sequences are available from Genbank: P100 is available under

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[1] as opposed to temperate/lysogenic phage which are not effective for removing their target bacteria

[2] R.M. Carlton *et al. / Regulatory Toxicology and Pharmacology 43 (2005) 301–312 (*"Bacteriophage P100 for control of *Listeria monocytogenes* in foods: Genome sequence, bioinformatic analyses, oral toxicity study, and application") (published after the priority date of this patent application).

[3] Loessner & Busse (*Appl Envir Microbiol.* June 1990) ("Loessner I") or Loessner *et al.* (*Appl Envir Microbiol.* 1996) ("Loessner II")

accession #DQ004855, and A511 under accession #DQ003638. Also, as stated above, both phages are deposited at the ATCC.

5. The skilled scientist in this field will know how to produce P100 phage by routine methods, if, for example, he starts with the deposited material. Our presently preferred method for producing P100 for use according to the present invention exploits a unique method developed after the filing date of this application by me and co-inventor Carlton for preparing high titre phage lysates that is based on the particular combination of P100 and its specific, non-pathogenic, host strain of *L. monocytogenes*. However, conventional methods known prior to our priority filing date may nevertheless be used routinely to produce P100 lysates.

6. It is noteworthy that no experimental proof exists for <u>any</u> of the assertions made in the Day patent with respect to the successful use of *Clostridia* phages in cheese or dairy products (or, as far as I know, any contaminated products). *Clostridia* phages are very tricky to deal with, as I know from direct personal experience. (For example, my group was the first to publish a sequence of a *Clostridium* phage; Zimmer *et al.*, 2003[4]). Unquestionably, Day did not teach or suggest anything specific or helpful about *Listeria* phages, and most certainly, nothing related to P100.

7. Moreover, to the best of my knowledge and based on our own (thus far unsuccessful) attempts, no lytic bacteriophage for *Clostridium tyrobutyricum* has been described in the scientific literature (even though I presume they may exist), and I know of no currently available virulent phages for lysing this bacterial species. This represents yet a further barrier to the ability to develop phages for use against *Clostridia* and emphasizes how far the Day patent really was from achieving its asserted objectives.

8. It is also important to consider the life cycle of *Clostridia* when considering Day in relation to the present invention. *Clostridium* bacteria can only initiate growth by first germinating from their spores and require an anaerobic environment for cell growth and multiplication. Only then do these cells become sensitive to phage infection and killing. Such conditions rarely apply in the food industry, though one example is in the center part of large cheese large as Swiss Emmental and

[4] Zimmer M, Sattelberger E, Inman RB, Calendar R, Loessner MJ, .*Mol Microbiol. 50*:303-17, 2003 ('Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed + 1 translational frameshifting in structural protein synthesis")

Gruyere varieties. This occurs at a time point that is later in the cheese ripening process. At this point, however, the cheese matrix has solidified, so that any phage that may have been introduced would have become is immobilized and, therefore, useless. Thus, the life cycle of *Clostridia* along with the above facts lead to a conclusion that the *Clostridium* phages known at that time could **not** have been used effectively in cheese (the stated objective of Day), because:

(a) they are unable to infect spores, and,

(b) when Clostridial cells enter the vegetative growth phase (emerging from the spore stage), any phages present would have already been effectively "removed" by immobilization in the hardened cheese matrix.

Therefore, in my opinion, the Day patent would not enable anyone to use *Clostridia* phages for the purposes indicated, let alone suggest to an investigator in this field what to do about *Listeria* or to look to the Loessner-I or Loessner-II papers on various *Listeria* phages for ideas about how to accomplish the objectives of the present invention. More emphatically, and rather obviously, nothing in Day would have suggested to the skilled scientist to consider either

(i) the P100 phage (the existence of which was first disclosed years later in the present patent application), or

(ii) any combination of P100 with other phages and substances

for treating food, food processing equipment or food containers to rid them of *Listeria* or other non-*Listeria* organisms at the same time.

9. I also wish to address the rejection of the composition claims based on the Examiner's belief that the claimed phage compositions are, in fact, unmanipulated "products of nature." P100 (and A511) are virulent phages. This means they can only exist and multiply (and therefore survive beyond a short period) in the presence of adequate numbers of host bacteria. However, this does not mean that such phage can always or commonly be found where the host bacteria are present. In fact, this is a **rare** situation, and only occurs where the density of host bacteria is sufficiently high to support multiplication of the phage. As we have described, P100 was isolated from sewage, which contains extreme numbers of bacteria, including *Listeria*. Of course, the phage were not, and cannot be, present in such material in "isolated" form, as the claims state, but are part of a very complex **mixture** of bacteria, phages, and multiple other types of organisms. We have never found, and, to my knowledge, no one else has reported, such phage as P100, A511 (or other lytic phage specific for other bacterial genera) to be naturally present in, or associated with,

any food product, most likely because of an inadequate supply of host bacteria. Therefore the Examiner's statements that

> *The invention as claimed, reads on the natural products, (i.e. phage P100 alone and in combination with phage A511, or other phage) that are substantially unaltered, and encompass compositions of food products (for example, cheese, dairy products, etc.) that are found contaminated with pathogenic bacteria such as Listeria monocytogenes (and/or other bacterial contaminants).*
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> *The invention, as recited, does not provide any structural distinction in the product as claimed, and the product resulting from bacterial contamination of food or dairy products commonly occurring in nature"*

indicate a lack of appreciation of the above-stated facts. Granted that bacterial contamination of food or dairy products occurs commonly in "nature." So the **host bacteria** for P100 and A511 do occur naturally in such an environment. But that is not evidence of the presence of lytic phage in or on such food products. Indeed the claimed bacteriophage is **not** found naturally there. And because the phage is lytic, it could not be "hiding" inside the bacteria in the form of a lysogen (where the bacterial host incorporates a temperate phage genome in its DNA). So, to close this subject, the bacteriophage compositions, as claimed, cannot be considered to be "products of nature" but rather have been acted upon by our intervention in isolating (and characterizing) and propagating them to produce isolated and pure populations -- which is what the ATCC deposits and phage populations that would be propagated in the laboratory from these deposits -- represent.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Zurich, April 4, 2008	/Martin Loessner/
Date	Martin Loessner